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Neotypification and redescription of *Amanita preissii* (Basidiomycota), and reconsideration of the status of *A. griseibrunnea*

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Abstract

Davison, E.M., Guistiniano, D., McGurk, L.E., Watkin, E.L.J. & Bougher, N.L. Neotypification and redescription of *Amanita preissii* (Basidiomycota), and reconsideration of the status of *A. griseibrunnea*. *Nuytsia* 28:193–204. *Amanita preissii* (Fr.) Sacc. is redescribed. Re-examination of collections of *A. griseibrunnea* O.K.Mill. show that they do not differ significantly from *A. preissii* and the two species are combined. This species is common in the Perth IBRA subregion. Sequence data from the nuclear ribosomal internal transcribed spacer (ITS) region, 28S nuclear ribosomal large subunit rRNA (28S) region, RNA polymerase II (RPB2) region, β-tubulin region and translation elongation factor 1-α (EF1-α) region have been deposited in GenBank.

Introduction

Amanitas are some of the most common and conspicuous mushrooms in the south-west of Western Australia, but their ephemeral fruiting and lack of clearly defined macroscopic characters mean that their taxonomy is poorly understood. Characterisation of the large number of local species has been assisted by the use of DNA sequences, together with detailed microscopic examination. As local interest in macrofungi has been sporadic, there has been little continuity of mycological knowledge in Western Australia and there is often uncertainty about the identity of described species.

Amanita preissii (Fr.) Sacc. is one of the first mushrooms described from the south-west of Western Australia. It was collected by Ludwig Preiss between 1839 and 1841 (*Preiss* 2665) and sent to Elias Fries for naming (Hilton 1982). The majority of Preiss' cryptogams were lodged at the Berlin Herbarium but were destroyed when it was burnt down following an air raid in the Second World War (Hilton 1982, 1988). No duplicate of *Preiss* 2665 is known. Fries' description, as translated by Gentilli (1953), is brief:

'*Agaricus (Amanita) Preissii* Fr., *cap* fleshy, convex-expanded, sticky, edge even; *stem* stuffed, mealy, pallid, rooting with turnip-shaped *volva* narrowed at the top, with a free persistent edge, *ring* placed

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high on the stem and pendulous, *gills* adnate, crowded. In sandy places in forests, May. Preiss's Herb., No. 2665.'

'A very noble species, clearly distinguished from all other known species by the nature of the volva. The size is that of *A. porphyrius* but the species should rather be compared with *A. ovoideus* and *A. solitarius*.'

Gentilli (1953) suggested that Preiss' original collection was made near Perth, and redescribed the most common amanita in Kings Park (a large reserve of native vegetation close to the centre of Perth) as *A. preissii* (Figure 1). His macroscopic description is given below because he may have been confusing more than one species:

'Cap 2–3 cm. in diameter when at the button stage, almost hemispherical (1a), gradually expanding to 5–8 cm., convexo-expanded (1e) and finally slightly depressed at the centre when adult (1g), slightly viscid in wet weather, usually smooth (1g), but at times with irregular flat warts which are hardly noticeable (1h), especially when the cap is loaded with soil (as is nearly always the case), colour white with a faint biscuit tinge towards the centre, the white always rather cream or ivory like, never chalky. A specimen with a pale salmon-coloured cap probably belongs to this species[1]. The cap is of medium thickness, always definitely fleshy (1g).'

'Gills adnate to adnexed, moderately crowded, straight at first (1h) then ventricose (1g), white at first, tending to creamy colour later on.'

'Stem 8–12 cm. high from its junction with the cap to its lower end. Over a length of 5 to 8 cm. from the top the stem is subcylindrical, tapering slightly upwards, with a diameter of 8 to 18 mm. at the top and 8 to 24 mm. at the bottom of the subcylindrical portion (stem proper). Below this is the bulbous portion, more or less turnip-shaped, nearly always with a pointed apex, 3–5 cm. high and with a maximum diameter of 22 to 43 mm., usually located between the upper third and upper half. The surface of the stem is always more or less mealy. Two specimens had rust-coloured marks on the stem.'

'Volva as a distinct upper edge to the bulbous portion of the stem, initially as a free membranous border which in one specimen extended up to 11 mm. upwards but was already torn (1h). In a few specimens the volva extends only for 2 to 5 mm. upwards (1j), and in many specimens no volva is visible even in the young stages (1a, c). If the stem is sectioned the place where the volva has been is usually marked by a sharp edge (1d). The volva is always membranous.'

Joseph Gentilli sent material to Cornelius Bas at Leiden who added microscopic details to Gentilli's description, including the amyloid elongate to cylindrical spores, the remnants of the universal veil on the pileus as abundant hyphae and rather abundant globose, ellipsoid and clavate inflated cells, and absence of clamp connections (Bas 1969). Bas (1969) also described *A. ochroterrea* Gentilli ex Bas from Kings Park from specimens that had been sent by Gentilli as *A. preissii* forma *ochroterrea*. In addition to these two species, Gentilli (1953) described other amanitas from Kings Park but the names were invalidly published (Reid 1980). No herbarium material from any of Gentilli's collections from Kings Park has been located in Western Australia. We have not examined Gentilli's collections known to be at L. *Amanita preissii* is believed to be poisonous (Harris & Stokes 1976).

Recent surveys of the macrofungi of Kings Park have recorded at least 13 *Amanita* Pers. species (Bougher 2010, 2011) and one of these fits the description of *A. preissii* (Figure 2). It is widespread in the Perth

[1]This may be a reference to *Amanita fibrillopes* O.K.Mill. that is known to occur in Kings Park.

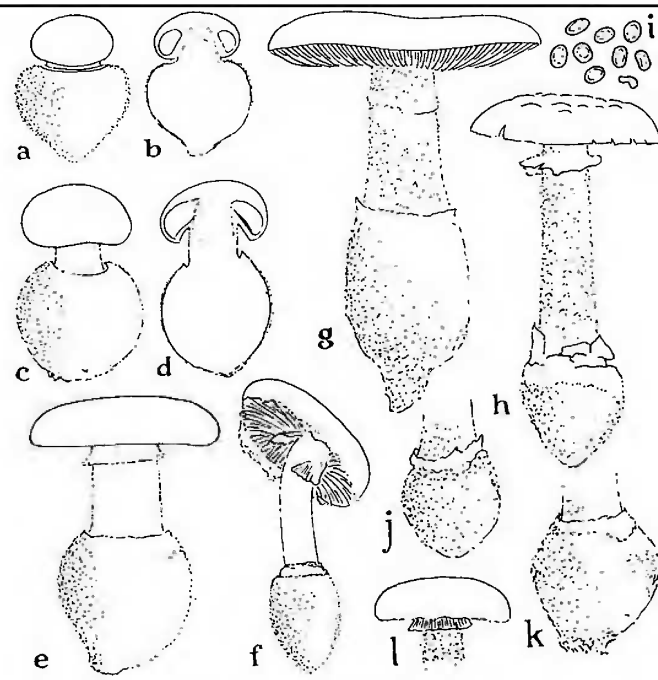

Fig 1.—*Amanita preissii* Fries.

(*a*) Very young specimen and (*b*) section of same. (*c*) Young specimen with cap well raised and distinct volval rim. (*d*) Section of same showing thickness of partial veil below the gills, and volval rim. (*e*) Young specimen with veil. (*f*) Young specimen with torn ring and cortina remnants. (*g*) Adult specimen with evanescent ring. (*h*) Adult specimen with unusually large ring and volval remnants, and warts on cap. (*i*) Spores, x 300. (*j*) and (*k*) Typical rooting portions of stems showing volval remnants. (*l*) Young specimen showing shape of cap, striated ring, and mealiness of stem.

—Illustrations half natural size throughout, excepting those of spores.

Figure 1. *Amanita preissii*. From Gentilli (1953), reproduced with permission.

Figure 2. *Amanita preissii*. Neotype specimens, photographed prior to preservation. Voucher: *N.L. Bougher* NLB 1105 (PERTH 08690766). Photograph by N.L. Bougher.

area, as noted by Gentilli (1953). Following detailed studies of this species, we nominate a collection from Kings Park as the neotype, and provide a detailed description.

Miller (1991) described *A. griseibrunnea* O.K.Mill. from collections made in Kings Park and from the grounds of Murdoch University. The appearance of *A. griseibrunnea* (Figures 3) is similar to that of *A. preissii* (Figures 1 & 2); however, in the protologue *A. griseibrunnea* is described as having a pileus that is orange-grey darkening to brownish grey, colours that are not apparent in photographs of the fresh collections. Their macroscopic and microscopic characters are similar, given the variation that occurs between collections of the same species. On this basis we synonymise *A. griseibrunnea* with *A. preissii*.

Molecular sequences are increasingly important in species descriptions. Schoch *et al.* (2012) have shown that the nuclear ribosomal internal transcribed spacer (ITS) region is an informative DNA barcode marker for fungi due to the high variability within this region; it has the highest probability of separating intra- and inter-specific samples of the broadest range of species. In their comparison of four gene regions they found that within the Basidiomycota, the ITS had most resolving power (0.79) for species discrimination, closely followed by the 28S nuclear ribosomal large subunit rRNA gene (28S) (Schoch *et al.* 2012). Other gene regions that have been used for *Amanita* spp. are β-tubulin, elongation factor 1-α (EF1-α) and RNA polymerase II (RPB2) (Cai *et al.* 2014). Within the *A. preissii* collections sampled, we have found that the ITS region is very variable, and that 28S, β-tubulin, EF1-α and RPB2 show greater similarity between collections.

Methods

Methodology is largely based on that of Tulloss (*c.* 2000); colours, including the colour of spores in deposit and other shades of white to cream (designated by letters A–G) are from Royal Botanic Garden, Edinburgh (1969) while codes for other colours are from Kornerup and Wanscher (1978). In the descriptions of basidiospores (and basidia) the notation [x/y/z] denotes x basidiospores measured from y basidiomes from z collections. Biometric variables for spores follow Tulloss (2012), i.e. '**L** = the average spore length computed for one specimen examined and the range of such averages, **L′** = average spore length computed for all spores measured, **W** = the average spore width computed for one specimen examined and the range of such averages, **W′** = average spore width computed for all spores measured, Q = the length/breadth for a single spore and the range of the ratio of length/breadth for all spores measured, **Q** = the average value of Q computed for one specimen examined and the range of such averages, **Q′** = the average value of Q computed for all spores measured'.

DNA extraction, ITS amplification, cloning and sequence analysis follow the methodology in Davison *et al.* (2013). Methodology for other gene regions is described below.

The 28S region was amplified with primers LROR and LR5 (Moncalvo *et al.* 2000). RPB2 was amplified with amanita specific primers Am-6 F and AM-7 R (Cai *et al.* 2014). β-tubulin was amplified with amanita specific primers Am- β-tubulin F and Am- β-tubulin R (Cai *et al.* 2014). EF1-α was amplified with primers 1-α 526F and 1567R (Rehner 2001). PCR amplification of 28S, RPB2, β-tubulin and EF1-α were conducted in a total volume of 10 µl reactions containing 1 × PCR polymerization buffer (MyTaq Reaction Buffer, Bioline) (containing 1 mM dNTP's, 3 mM $MgCl_2$, stabilizers and enhancers), 0.1–0.25 µM of forward and reverse primer and 5 µm *Taq* Polymerase (Bioline). Reactions were performed in triplicates and pooled for sequencing to reduce sequencing errors.

All PCR reactions were performed in a Veriti™ thermocycler (Thermo Fisher Scientific) with the following parameters: initial denaturation of 95°C for 5 min followed by 35 cycles of 95°C denaturation for 20 s, 54°C (RPB2, β-tubulin) or 56°C (28S, EF1-α) annealing for 20 s, 72°C elongation for 1 min, with a single final elongation at 72°C for 5 min. PCR products were visualized by gel electrophoresis (1% agarose gel pre-stained with GelGreen (1 µl GelGreen per 100 ml of melted agarose, Biotum), 1 × Tris Acetic acid EDTA (TAE), run at 100 volts) to confirm amplification. PCR product was sent for sequencing using the commercial services of Australian Genome Research Facility. Sequence data was assembled with Geneious version 6.0.5 created by Biomatters (2016).

Phylogenetic trees of the 28S gene region were built using MEGA version 5 (Tamura *et al.* 2011). The Maximum Likelihood method was used, using a General Time Reversible model with gamma distributed rates plus invariant sites. A bootstrap consensus tree was inferred from 500 replicates.

Sequences have been deposited in GenBank; sequence identifiers and voucher information are given under each collection in this paper. The sequences were used as queries for NCBI nucleotide database using BLASTn (National Library of Medicine 2017).

Taxonomy

Amanita preissii (Fr.) Sacc. *Syll. Fung.* 5: 9 (1887). *Agaricus preissii* Fr., *Pl. Preiss.* 2: 131 (1846). *Type*: 'In arenosis umbrosis sylvarum' [Western Australia], May [1839–1841], *L. Preiss* 2665 (*holotype*: B *n.v.*, destroyed in WWII). *Neotype*: Kings Park, Western Australia [precise locality withheld for conservation reasons], 25 June 2015, *N.L. Bougher* NLB 1105 (*neotype*, here designated: PERTH 08690766!) [MB171396]. ITS sequences GenBank KY290657–KY290661; 28S sequence GenBank KY290654; RPB2 sequence GenBank KY288484; β-tubulin sequence GenBank KY273105; EF1-α sequence GenBank KY273109.

Amanita griseibrunnea O.K. Mill., *Canad. J. Bot.* 69(12): 2693 (1992), *syn. nov. Type*: Murdoch University forest, Western Australia, 7 May [originally published as June] 1989, *O.K. & H.H. Miller; E. & P. Davison* OKM 23629 (*holotype*: PERTH 02224518!) [MB517339].

Pileus 25–82 mm wide, to 6 mm thick, white to ivory white (B) aging saffron (4B2–5B2–B4), without surface staining or bruising, initially convex becoming plane with depressed centre and decurved margin, surface dry; margin non-striate, appendiculate with cream (C) to pale saffron to pale cinnamon (4B2–6B2) floccules from partial veil adhering. *Universal veil on pileus* adnate, felted to floccose, sticky, as small, thin patches in centre of disc, white aging cream (F) to saffron (5A3–B4). *Lamellae* adnexed to adnate, subcrowded, white to ivory white (B), to 12 mm broad, margin concolorous, fimbriate; *lamellulae* frequent in several lengths, shortest truncate, longest attenuate. *Stipe* 40–90 mm long, 8–18 mm wide, cylindrical or tapering upwards, white to ivory white (B), surface below partial veil floccose to scurfy with ornamentation white to ivory white (B) bruising saffron (4B2–5B2). *Partial veil* superior, descendent, fragile with margin ragged, flaring or adpressed, striate above, white to ivory white (B) to saffron to pale cinnamon (4B2–6B2). *Bulb* 20–40 × 15–35 mm, napiform to ovoid with tapered base, white to ivory white (B) bruising yellow. *Remains of universal veil* at top of bulb as a fragile, easily detached free limb or as a narrow rim or as concentric bands on the stipe or as flat warts, white or ivory white (B) bruising yellow. *Pileus and stipe context* white to ivory white (B), yellowing in stipe, stipe solid becoming hollow. *Smell* chemical, chlorine. *Spore deposit* white becoming cream (C) with age. (Figures 1–3)

Basidiospores [340/11/11] (7.5–)8.5–11.5(–12.5) × 5–6.5(–7.5) µm (**L** = 8.8–11.2 µm; **L'** = 9.7 µm; **W** = 5.2–6.0 µm; **W'** = 5.6 µm; **Q** = (1.11–)1.43–2.09(–2.27); **Q** = 1.65–2.13; **Q'** = 1.75), colourless, thin-walled, smooth, amyloid, elongate or cylindrical, occasionally ellipsoid, contents monogutullate; apiculus sublateral, cylindrical, *c.* 1 × 1 µm, truncate. *Pileipellis* not clearly differentiated in young specimens, to 180 µm thick in old specimens, colourless or pale yellow in NH_4OH; filamentous hyphae 2–10 µm wide, colourless, with gelatinising walls with widest constricted at septa, radially orientated with some interweaving; inflated cells not observed; vascular hyphae 2–10 µm wide, occasionally branched, very infrequent; clamp connections not observed. *Pileus context* of dominant or equal filamentous hyphae 3–35 µm wide, with widest constricted at septa, thin-walled, colourless; inflated cells to 220 × 40 µm when clavate, to 250 × 20 µm when cylindrical, to 250 × 35 µm when ventricose, colourless; vascular hyphae very infrequent, 3–10 µm wide, pale yellow; clamp connections not observed. *Lamella trama* bilateral, divergent. *Central stratum* of thin-walled, colourless, filamentous hyphae 3–10 µm wide; inflated cells not observed; vascular hyphae not observed; clamp connections not observed. *Subhymenial base* with angle of divergence 10°–15° from central stratum with filamentous hyphae following smooth broad curve to subhymenium, of dominant thin-walled, colourless, frequently branched filamentous hyphae 3–20 µm wide, widest proximal to subhymenium and constricted at septa; inflated cells frequent, colourless, to 100 × 20 µm, clavate, ellipsoidal, ventricose or cylindrical, terminal or intercalary; vascular hyphae very infrequent, 3–5 µm wide, pale yellow; 1 clamp connection observed. *Subhymenium* with basidia arising terminally from barely inflated to inflated hyphal segments to 15 µm wide; clamp connections not observed. *Lamella edge tissue* sterile, with frequent to infrequent inflated cells clavate or pyriform or cylindrical, 15–55 × 7–20 µm, colourless, disarticulating; clamp connections not observed. *Basidia* [320/11/11] (33–)37–60(–67) × (9–)10–14(–16) µm, thin-walled, colourless, *c.* 80% 4-spored, *c.* 10% 3-spored, *c.* 8% 2-spored, *c.* 2% 1-spored, sterigmata to 7 × 2 µm; 1 clamp connection observed. *Universal veil on pileus* merging into pileipellis, not layered, with elements irregularly disposed; filamentous hyphae 3–10 µm wide, colourless, gelatinising; inflated cells dominant, to 100 × 100 µm when spherical, to 60 × 50 µm when ovoid, to 90 × 60 µm when pyriform, to 110 × 35 µm when clavate, intercalary or in terminal chains of up to 3 cells, colourless or occasionally with pale brown contents, gelatinising; vascular hyphae very infrequent, 3–6 µm wide, pale yellow; clamp connections not observed. *Universal veil on stipe base* without clear orientation; filamentous hyphae dominant or equal, 3–20 µm wide, colourless or occasionally with pale brown contents, gelatinising; inflated cells to 110 × 80 µm ovoid, to 60 × 60 µm spherical, to 95 × 60 µm pyriform, to 110 × 30 µm clavate, intercalary or in terminal chains of up to 2 cells, colourless or occasionally with pale brown contents, gelatinising; vascular hyphae very infrequent, 2–3 µm wide, pale yellow; clamp connections not observed. *Stipe context* longitudinally acrophysalidic; filamentous hyphae 2–12 µm wide, colourless; acrophysalides dominant, to 600 µm long × 40 µm wide, clavate, terminal, colourless, gelatinising; vascular hyphae infrequent to frequent, 3–13 µm, brownish yellow, often sinuous; clamp connections not observed. (Figure 4)

Diagnostic features. Small to medium fruiting bodies with a white or ivory white pileus that ages saffron. The universal veil is thin and sticky, forming small patches in the centre of the disc. The pileus is initially white but ages cream to saffron. There are often cream to saffron to pale cinnamon floccules from the ring attached at the margin of the pileus. The gills are white to ivory white; the stipe is covered with white to ivory white floccose ornamentation that bruises saffron. The soft, fragile ring is superior, initially white, but becomes cream to saffron to pale cinnamon with age. The bulb is ovoid with a tapered base and bruises yellow. Old fruiting bodies have a strong chlorine odour. The spores are elongate or cylindrical and amyloid. The universal veil on the pileus has elements with no dominant orientation and is predominantly composed of inflated cells that may be in short chains. Clamp connections are absent.

Figure 3. *Amanita griseibrunnea*. Type specimens from Murdoch University forest, photographed prior to preservation. Voucher: *O.K. & H.H. Miller; E. & P. Davison* OKM 23629 [E 506] (PERTH 02224518). Photograph by N.L. Bougher.

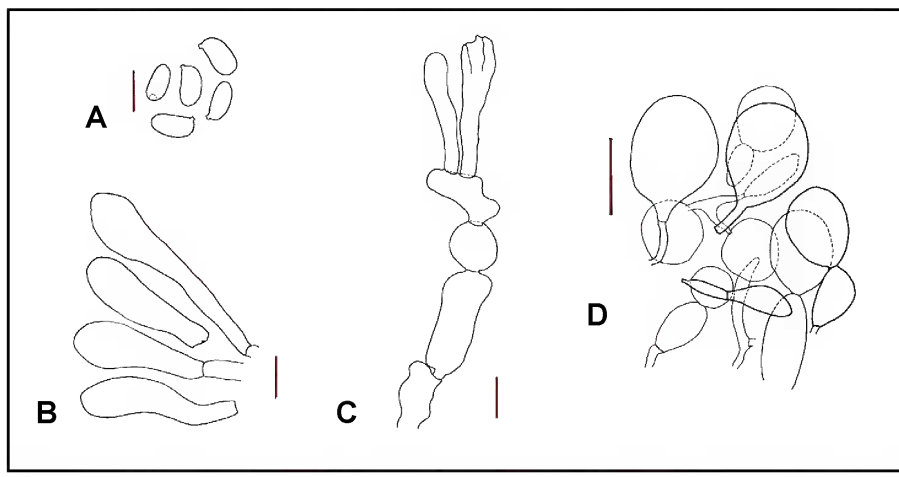

Figure 4. *Amanita preissii*. A – spores from print; B – lamella edge cells; C – squash of basidia and subhymenium; D – scalp section of universal veil on pileus. Scale bars = 10 µm (A–C); 50 µm (D). Line drawings from *N.L. Bougher* NLB 1105 (PERTH 08690766).

Selected specimens examined. WESTERN AUSTRALIA: [localities withheld for conservation reasons] 18 June 1995, *E.M. Davison* 7-1995 *& P.J.N. Davison* (PERTH, ITS GenBank JX398317, KY290667–KY290671, 28S GenBank KY290656); 2 July 1995, *E.M. Davison* 15-1995 *& P.J.N. Davison* (PERTH, ITS GenBank JX398318, β-tubulin GenBank KY273106, EF1-α GenBank KY273107); 13 June 2004, *E.M. Davison* 2-2004 *& P.J.N. Davison* (PERTH); 30 June 2009, *E.M. Davison* 3-2009 *& P.J.N. Davison* (PERTH, ITS GenBank KY290672–KY290676, 28S GenBank KY290655); 22 May 2005, *L.E. McGurk* 2005-35 LM (PERTH, ITS GenBank JX398322, KY290662–KY290666, EF1-α GenBank KY273108, RPB2 GenBank KY288485); Lightening Swamp, 2007, *L.E. McGurk* 24-2007 (PERTH); 7 May 1989, *O.K. Miller* 23623 (PERTH); 21 May 1989, *O.K. Miller* 23663 *& H. Miller* (PERTH).

Fruiting period. April to July.

Distribution and habitat. Solitary to gregarious in sandy soil and lateritic gravel, in native vegetation; nearby plants include *Allocasuarina fraseriana*, *Acacia pulchella*, *Corymbia calophylla*, *Callitris* sp., *Eucalyptus gomphocephala*, *E. marginata*, *Macrozamia fraseri* and *Pinus pinaster*. Occurs in the Swan Coastal Plain SWA2 Perth and JAF01 Northern Jarrah Forest IBRA subregions (as defined in Department of the Environment 2013).

Conservation status. To be listed as Priority Three under Department of Parks and Wildlife Conservation Codes for Western Australian Flora (M. Smith pers. comm.).

Suggested common name. Cinnamon-ring Lepidella.

Affinities based on molecular sequences. The ITS region is used as a barcode marker for fungi with a resolving power of 0.79 within the Basidiomycota (Schoch *et al.* 2012). We have, however, found that this region is very variable in the *A. preissii* collections sequenced. This region is 631 base pairs long in our analysis, and differences between 23 cloned sequences from five collections from three locations are from 0.16–3.65%. There are no exact matches on GenBank. The closest sequence is KP137085 *Amanita* sp. AD-C55022 clone 12_3, with 94% similarity and 100% query coverage.

Schoch *et al.* (2012) found that the 28S gene has better resolving power in some taxonomic groups and we have found that sequences of this gene region from different collections are much more similar. A Maximum Likelihood tree based on a subset of the 28S gene sequences used by Li *et al.* (2016) shows that *A. preissii* falls within *A.* sect. *Lepidella* (E.-J.Gilbert) Corner & Bas clade III (Figure 5). The closest sequence is HQ539749 *A. sublutea* (Cleland) E.-J. Gilbert PSC 2401 from South Australia.

The β-tubulin partial sequence is 380 base pairs long in our analysis. There are no differences between the sequences obtained from two collections from two locations. There are no exact matches on GenBank. The closest sequence is KJ466517 *A. modesta* Corner & Bas HKAS75405 with 90% similarity and 89% query coverage.

The EF1-α partial sequence is 511 base pairs long in our analysis. The differences between sequences obtained from three collections from three localities are 0.00–0.18%. There are no exact matches on GenBank. The closest sequences are KJ481996 *A. virgineoides* Bas HKAS79691, KJ481943 *A. manginiana* Har. & Pat. HKAS56933 and KJ481945 *A. modesta* HKAS75405 all with 87% similarity and 96% query coverage.

The RPB2 partial sequence is 598 base pairs long in our analysis. There is no difference between two sequences obtained from two collections from different localities. There are no exact matches on

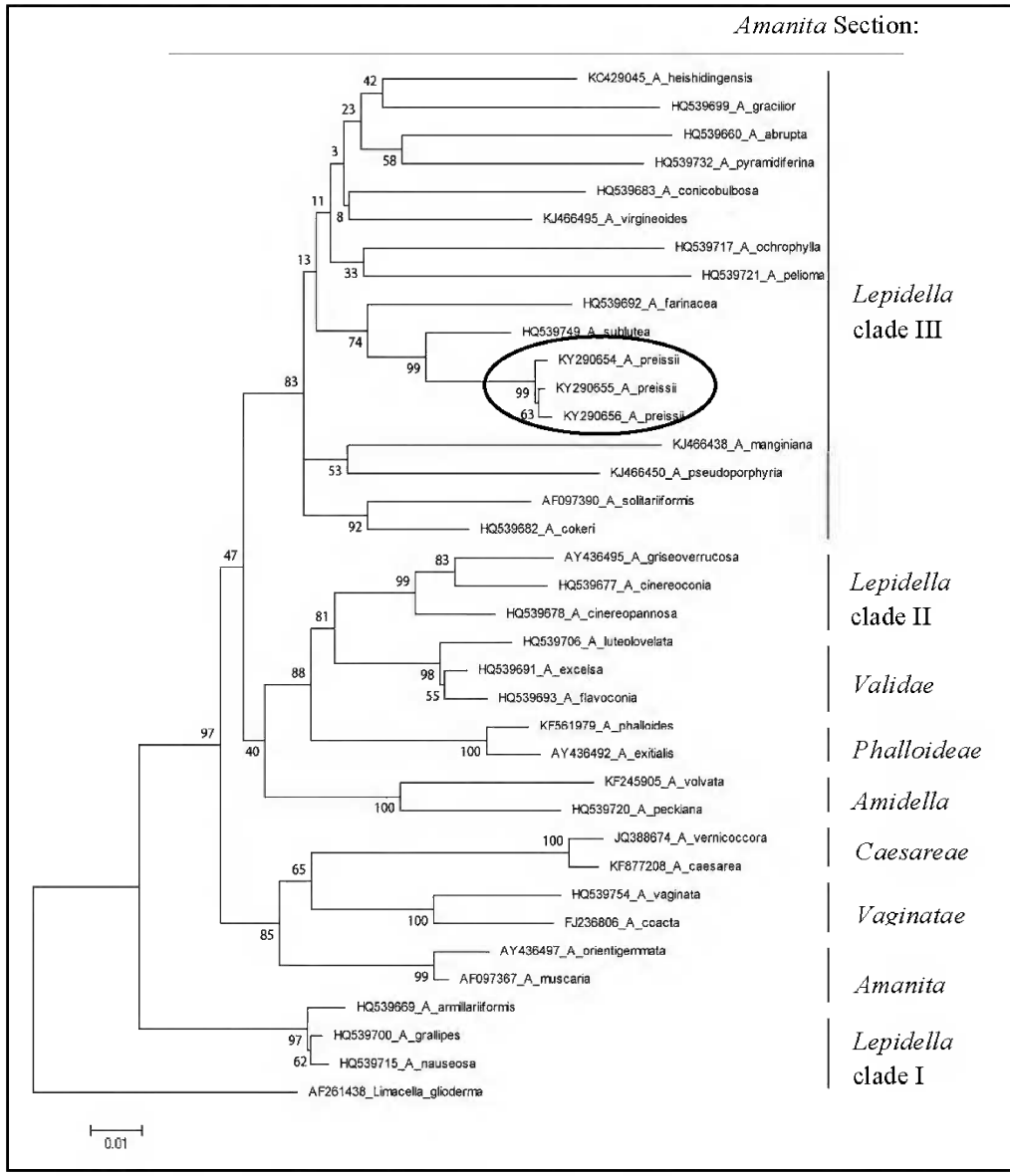

Figure 5. Molecular phylogenetic analysis by Maximum Likelihood method of 28S sequences showing the position of *Amanita preissii* in *A.* sect. *Lepidella* clade III. Bootstrap support values are shown above the nodes. The tree is rooted on *Limacella glioderma* (Fr.) Maire. Reproduced from Li *et al.* (2016).

GenBank. The closest sequences are KJ466605 *A. modesta* HKAS75405 with 91% similarity and 100% query coverage and KJ466606 *A. oberwinklerana* Zhu L. Yang & Yoshim. Doi HKAS77330 with 91% similarity and 99% query coverage.

Notes. The distinguishing characters of *A. preissii* are given by Bas (1969) and Reid (1980), with the macroscopic characters based on Gentilli (1953). Bas (1969) placed *A. preissii* in *A.* sect. *Lepidella* stirps *Preissii* based on its overall size and colouration, volval limb, spore shape and size, and basidia without clamp connections. He distinguished it from other species in this stirps because of the absence

of yellow bruising in the context, by the shape of the bulb, spore shape and size, abundance and shape of inflated cells in the universal veil on the pileus, and occurrence in Western Australia. The description by Reid (1980) is based on that of Bas (1969). Reid (1980) distinguishes *A. preissii* from other Australian species on the basis of pileus colour, overall size, spore shape and absence of clamp connections. The collections described in this paper fit the descriptions given by Gentilli (1953), Bas (1969) and Reid (1980).

Miller's description of *A. griseibrunnea* (Miller 1991) differs from that of Gentilli (1953), Bas (1969) and Reid (1980) in the colour of the pileus which he describes as 'orange-grey (5B2), darkening to brownish grey (5B2 to 5C2)'. However the photograph of the *A. griseibrunnea* type (which was not published) shows a white or pale pileus that either does not have, or only has a very slight tint of orange-grey (Figure 3). *Amanita preissii* develops these colours with age (Figure 2). Other macroscopic characters are similar.

Microscopic characters are also similar. The spores of the three *A. griseibrunnea* collections are of similar size and shape to two collections of *A. preissii* from Kings Park (the site of Gentilli's collections and one of the sites from which *A. griseibrunnea* was described) (*A. griseibrunnea* [60/3/3] 9–12(–12.5) × 5–6.5 µm (**L** = 9.8–11.2 µm; **L'** = 10.8 µm; **W** = 5.3–6.0 µm; **W'** = 5.6 µm; Q = (1.58–)1.64–2.27; **Q** = 1.74–2.13; **Q'** = 1.92), *A. preissii* (Kings Park collections PERTH 08690766, PERTH 08774765) [40/2/2] 9–11(–12) × 5–6 µm (**L** = 10–10.6 µm; **L'** = 10.3 µm; **W** = 5.2–5.5 µm; **W'** = 5.3 µm; Q = (1.64–)1.80–2.10(–2.20); **Q** = 1.92–1.95; **Q'** = 1.94)). The universal veil on the pileus and at the stipe base is of similar structure in both species and is composed of dominant or equal inflated cells that are terminal or in short chains of two, and of spherical, ellipsoid, ovoid, clavate or pyriform shape (Figure 6).

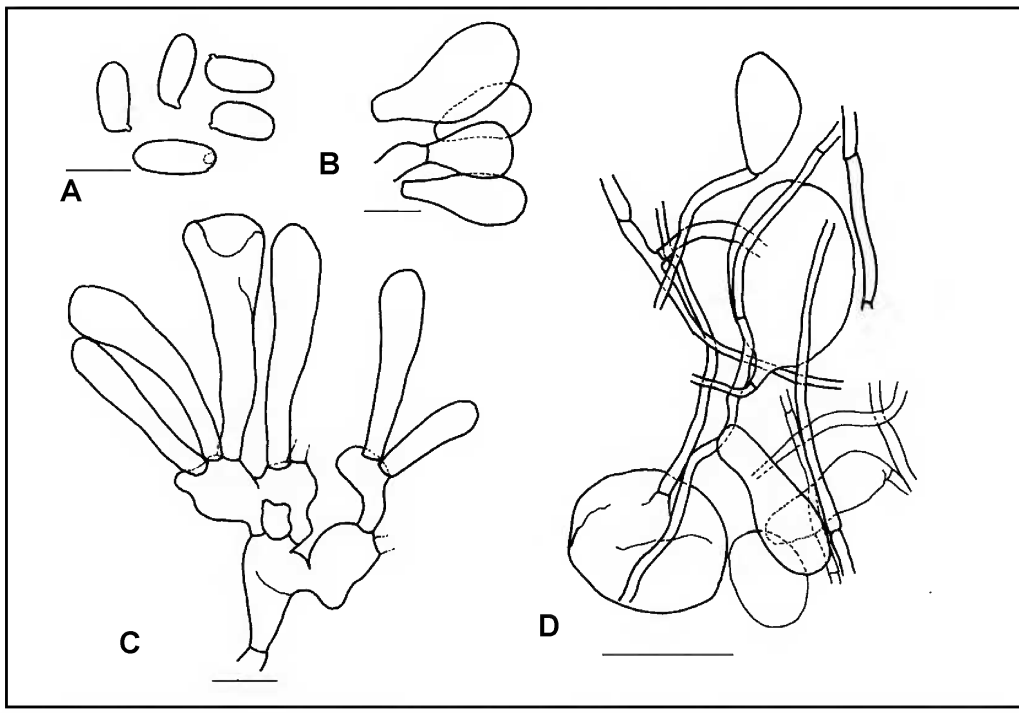

Figure 6. *Amanita griseibrunnea* type. A – spores from print; B – lamella edge cells; C – squash of basidia and subhymenium; D – squash of universal veil on pileus. Scale bars = 10 µm (A–C); 50 µm (D). Line drawings from *O.K. & H.H. Miller; E. & P. Davison* OKM 23629 [E 506] (PERTH 02224518).

The type locality for *A. griseibrunnea* is the grounds of Murdoch University, where *A. preissii* is common. The other locality where it was collected is Kings Park, where *A. preissii* also occurs.

Attempts to obtain usable DNA from *A. griseibrunnea* (PERTH 02224518) have not been successful.

On the basis of its appearance, apart from the colour of the pileus (which is not supported by contemporary photographs), microscopic characters and locations where it was collected, *A. griseibrunnea* is synonymised with *A. preissii*.

In his comments about *A. griseibrunnea*, Miller (1991) makes no mention of *A. preissii* even though he was aware of its redescription by Gentilli (1953) and Bas (1969). Miller considered that there were six Western Australian taxa in *A.* sect. *Lepidella* stirps *Preissii* which formed a complex (Miller & Davison 1994). He examined Gentilli's collections of *A. preissii* in L in 2003 but took the matter no further (O.K. Miller pers. comm.).

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References

Bas, C. (1969). Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella*. *Persoonia* 5: 285–579.

Biomatters (2016). Biomatters development team. Copyright 2005–2016 Biomatters Ltd. http://www.geneious.com

Bougher, N.L. (2010). History of the study of fungi at Kings Park, Western Australia. *The Western Australian Naturalist* 27: 61–90.

Bougher, N.L. (2011). New records of fungi and slime moulds at Kings Park, Perth, Western Australia. *The Western Australian Naturalist* 28: 24–42.

Cai, Q., Tulloss, R.E., Tang, L.P., Tolgor, B., Zhang, P., Chen, Z.H. & Yang, Z.L. (2014). Multi-locus phylogeny of lethal amanitas: implications for species diversity and historical biogeography. *BMC Evolutionary Biology* 14: 143.

Davison, E.M., McGurk, L.E., Bougher, N.L., Syme, K. & Watkin, E.L.J. (2013). *Amanita lesueurii* and *A. wadjukiorum* (Basidiomycota), two new species from Western Australia, and an expanded description of *A. fibrillopes*. *Nuytsia* 23: 589–606.

Department of the Environment (2013). *Australia's bioregions (IBRA)*, IBRA7, Commonwealth of Australia. http://www.environment.gov.au/land/nrs/science/ibra#ibra [accessed 2 January 2017].

Gentilli, J. (1953). Amanitas from Kings Park, Perth. *The Western Australian Naturalist* 4: 25–34, 59–63.

Harris, A.R.C. & Stokes, J.B. (1976). *Amanita preissii* "mushroom" poisoning. *The Medical Journal of Australia* 2: 568–571.

Hilton, R.N. (1982). A census of the larger fungi of Western Australia. *Journal of the Royal Society of Western Australia* 65: 1–15.

Hilton, R.N. (1988). The Preiss collection of Western Australian fungi. *Nuytsia* 6: 295–304.

Konerup, A. & Wanscher, J.H. (1978). *Methuen handbook of colour*. (Methuen: London.)

Li, G.J., Hyde, K.D., Zhao, R.L. *et al.* (2016). Fungal diversity notes 253–366: taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* 78: 1–237.

Miller, O.K. (1991). New species of *Amanita* from Western Australia. *Canadian Journal of Botany* 69: 2692–2703.

Miller, O.K. & Davison, E.M. (1994). Observations on the *Amanita preissii* complex in Western Australia. Fifth International Mycological Congress, Vancouver.

Moncalvo, J.M., Lutzoni, F.M., Rehner, S.A., Johnson, J. & Vilgalys, R. (2000). Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. *Systematic Biology* 49: 278–305.

National Library of Medicine (2017). http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome [accessed 10 August 2016].

Reid, D.A. (1980). A monograph of the Australian species of *Amanita* Pers. ex Hook. (Fungi). *Australian Journal of Botany, Supplementary Series* No. 8: 1–97.

Rehner, S. (2001). Primers for Elongation Factor 1-α (EF1-α). http://www.aftol.org/pdfs/EF1primer.pdf [accessed 10 December 2014].

Royal Botanic Garden, Edinburgh (1969). *Flora of British fungi: colours identification chart.* (Her Majesty's Stationery Office: Edinburgh.)

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W. & Fungal Barcoding Consortium (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* 109: 6241–6246.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2740.

Tulloss, R.E. (*c.* 2000). Notes on methodology for study of *Amanita* (Agaricales). http://www.amanitaceae.org/content/uploaded/pdf/methodsb.pdf [last accessed 11 August 2016].

Tulloss, R.E. (2012). Biometric variables: meanings and how to define a range. http://www.amanitaceae.org/?How%20to%27s [accessed 26 March 2013].